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14. ABSTRACT

Breast cancer is the most commonly occurring cancer among women. Many risk factors have been identified but a positive family history remains among the most important ones established for breast cancer. Mutations in the currently known high-risk breast cancer genes (such as BRCA1/2, etc) are common in familial breast cancer, but they can explain at best 20–25% of the overall excess familial risk and less than 5% of the total breast cancer incidence. It is most likely that residual genetic susceptibility is driven by variants at many loci, each conferring a moderately risk of the disease. A great deal of efforts has been put to identify novel moderate-risk breast cancer genes, but only a few have been identified and confirmed (3-7). Thus, there are still a great amount of unidentified risk alleles that confer susceptibility to breast cancer to be found. Some of them might be at unconventional loci (such as protein non-encoding genes) and traditionally overlooked regions (such as 3"UTR of mRNA). Differences exist between African American (AA) and Caucasian women in the incidence and nature of breast cancer (8-10). Although the breast cancer incidence rates are 20-40% higher in Caucasian women than in AA women, AA women are more likely to be diagnosed before age 50 and have aggressive diseases with poor prognosis, as characterized by a higher proportion of high-grade tumors, estrogen receptor negative (ER-)/progesterone receptor negative(PR-) tumors, compared with Caucasian women. Therefore, we hypothesize that genetic variations in miRNA genes, responsive elements in target genes and miRNA processing genes will modify breast cancer risk. To test these hypothesis we will estimate the frequencies of 35 SNPs in miRNA genes that predicted to regulate key breast cancer genes, and 22 SNPs in their responsive elements in target genes for breast cancer cases and controls. It is expected that adverse genotypes of miRNA genes and responsive elements in target genes are associated with an increased risk of breast cancer. We will estimate the frequencies of 67 tagSNPs in miRNA processing genes (Drosha, Dicer, DGCR8, XPO5, TRBP and AGO2) for breast cancer cases and controls. It is expected that adverse haplotypes of miRNA processing genes are associated with an increased risk of breast cancer. The study will further our understanding of the genetic events leading to the development of breast cancer; explore the genetic basis of miRNA in breast cancer; and eventually provide a means of identifying a subgroup that is most likely to develop breast cancer. Such individuals may then be targeted for specific intervention programs such as chemoprevention and dietary modification.

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INTRODUCTION

Breast cancer is the most commonly occurring cancer among women. Many risk factors have been identified but a positive family history remains among the most important ones established for breast cancer. Mutations in the currently known high-risk breast cancer genes (such as *BRCA1/2*, etc) are common in familial breast cancer, but they can explain at best 20–25% of the overall excess familial risk and less than 5% of the total breast cancer incidence. It is most likely that residual genetic susceptibility is driven by variants at many loci, each conferring a moderately risk of the disease. A great deal of efforts has been put to identify novel moderate-risk breast cancer genes, but only a few have been identified and confirmed (3-7). Thus, there are still a great amount of unidentified risk alleles that confer susceptibility to breast cancer to be found. Some of them might be at unconventional loci (such as protein non-encoding genes) and traditionally overlooked regions (such as 3"UTR of mRNA). Differences exist between African American (AA) and Caucasian women in the incidence and nature of breast cancer (8-10). Although the breast cancer incidence rates are 20-40% higher in Caucasian women than in AA women, AA women are more likely to be diagnosed before age 50 and have aggressive diseases with poor prognosis, as characterized by a higher proportion of high-grade tumors, estrogen receptor negative (ER-)/progesterone receptor negative(PR-) tumors, compared with Caucasian women. Therefore, we hypothesize that genetic variations in miRNA genes, responsive elements in target genes and miRNA processing genes will modify breast cancer risk. To test these hypotheses we will estimate the frequencies of 35 SNPs in miRNA genes that predicted to regulate key breast cancer genes, and 22 SNPs in their responsive elements in target genes for breast cancer cases and controls. It is expected that adverse genotypes of miRNA genes and responsive elements in target genes are associated with an increased risk of breast cancer. We will estimate the frequencies of 67 tagSNPs in miRNA processing genes (Drosha, Dicer, DGCR8, XPO5, TRBP and AGO2) for breast cancer cases and controls. It is expected that adverse haplotypes of miRNA processing genes are associated with an increased risk of breast cancer. The study will further our understanding of the genetic events leading to the development of breast cancer; explore the genetic basis of miRNA in breast cancer; and eventually provide a means of identifying a subgroup that is most likely to develop breast cancer. Such individuals may then be targeted for specific intervention programs such as chemoprevention and dietary modification.

BODY

Study population. The Women's Circle of Health Study (WCHS) was designed specifically to study the role of genetic and non-genetic factors in relation to aggressive breast cancer risk in AA and EA women. Study design, enrollment, and collection of data and biospecimens have been described in detail previously [1]. Briefly, women diagnosed with incident breast cancer were identified through both hospital-based case ascertainment in targeted hospitals that had large referral patterns of AAs in four boroughs of the metropolitan New York City area, and using population-based case ascertainment in seven counties in New Jersey (NJ) through the NJ State Cancer Registry. The eligibility criteria for cases were: self-identified AA and EA women, 20-75 years of age at diagnosis, no previous history of cancer other than non-melanoma skin cancer, recently diagnosed with primary, histologically confirmed breast

cancer, and English speaking. Controls without a history of any cancer diagnosis other than non-melanoma skin cancer living in the same area as cases were identified through random digit dialing and were matched to cases by self-reported race and 5-year age categories. Following agreement to participate, in-person interviews were conducted to complete informed consent and to query participants on a number of potential risk factors, including medical history, family history of cancer, diet, physical activity, and other lifestyle factors. Anthropometric measures were taken, and biospecimens were collected. Blood samples were initially collected, but due to logistical and cost constraints, we transitioned to saliva samples after enrollment of approximately 850 participants. Permission to obtain pathology data, including ER status, as well as tumor tissue blocks was included in the informed consent form. This study was approved by the Institutional Review Boards at Roswell Park Cancer Institute (RPCI), the Cancer Institute of New Jersey (CINJ) – Robert Wood Johnson Medical School (RWJMS), Mount Sinai School of Medicine (MSSM), and the participating hospitals in NYC. At the end of the study, DNA and data were completely available for 553 AA cases and 466 AA controls. We selected 383 EA cases and 382 EA controls from the WCHS by frequency matching them to AA cases and controls by 5-year age group.

Table 1 outlines the characteristics of the study population. AA women tended to have a higher BMI than EA women (31.4 vs 27.3 kg/m2), less likely to use HRT (86% non-users in AA cases and controls vs. 76% non-users in EA cases and controls), and tended to have lower frequencies of family history of breast cancer (13.4% vs 22.5%), when compared to EA cases. The majority of women had college and graduate school education, but rates of women who pursued higher education were lower in AAs (57.7%) vs. EAs (82.1%). As expected, family history of breast cancer was higher in the cases than controls for both AAs and EAs, and controls were more highly educated. Among EAs, HRT use was higher among cases, although the association was not statistically significant.

Table 1. Descriptive characteristics of African American and European American breast cancer cases and controls

Characteristics	African American			European American		
	Case (n=547)	**Control (n=461)**	**P***	**Case (n=381)**	**Control (n=382)**	**P***
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
Age	51.7 (10.0)	49.8 (9.9)	0.003	51.0 (8.4)	50.9 (8.3)	0.82
Body mass index	31.2 (6.7)	31.6 (7.8)	0.48	26.8 (5.8)	27.7 (7.1)	0.06
% European ancestry	0.09 (0.15)	0.10 (0.16)	0.19	0.98 (0.07)	0.99 (0.03)	0.07
	Count (%)	Count (%)		Count (%)	Count (%)	
Menopausal status			0.14			0.17
Premenopausal	337 (61.6)	263 (57.0)		235 (61.7)	217 (56.8)	
Postmenopausal	210 (38.4)	198 (43.0)		146 (38.3)	165 (43.2)	
Family history			0.13			0.001
Yes	82 (15.0)	54 (11.7)		104 (27.3)	67 (17.5)	
No	465 (85.0)	407 (88.3)		277 (72.7)	315 (82.5)	
Education			0.06			<0.001
Less than high school	76 (13.9)	55 (11.9)		9 (2.4)	4 (1.1)	
High school	175 (32.0)	122 (26.5)		80 (21.0)	44 (11.5)	
College and graduate school	296 (54.1)	284 (61.6)		292 (76.6)	334 (87.4)	
Hormone replacement therapy			0.74			0.47
Yes	79 (14.5)	62 (14.2)		96 (25.3)	88 (23.0)	
No	464 (85.5)	397 (85.8)		284 (74.7)	294 (77.0)	

Footnote: *P derived from student t-test for continuous variables and chi-square test or Fisher's exact test for categorical variables. *Abbreviation:* SD: standard deviation.

Specific Aim 1: *To investigate whether SNPs in miRNA genes that are predicted to regulate key breast cancer genes, and SNPs in responsive elements in these key genes, are associated with breast cancer risk in CA and AA women, and whether the association is different between CA and AA women.*

Specific Aim 2: *To assess whether haplotypes in miRNA processing genes (Drosha, Dicer, DGCR8, XPO5, TRBP and AGO2) are associated with breast cancer risk in CA and AA women, and whether the association is different between CA and AA women.*

Identification of SNPs. For this study, we focused on miRNA genes that are predicted to regulate key breast cancer genes (*BRCA1/2*, *p53*, *PTEN*, *CHEK2*, *ATM*, *NBS1*, *RAD50*, *BRIP1*, *PALB2*, *ER*, *PR* and *ERBB2*). A total of 146 miRNAs fit the criteria. We searched Entrez SNP (http://www.ncbi.nlm.nih.gov/ entrez/query.fcgi?CMD=search&DB=snp) for SNPs in pre-miRNA regions of these selected miRNAs and in binding sites of target genes and identified 99 SNPs with minor allele frequencies (MAFs) greater than 0.05 in either EAs or AAs. For miRNA processing genes, including *AGO1*, *AGO4*, *DGCR8*, *XPO5*, *PACT*, and *TARBP2*, we searched an extended genomic region 15kb from both 3' and 5' ends of each gene. Genotype data were downloaded from HapMap (23) and other resequencing projects through the Genome Variation Service at Seattle SNP (http://gvs.gs.washington.edu/GVS/), and multi-population tagSNPs to capture variations in both populations of European and African ancestry were selected using the TAGster program [2]. In total, 154 SNPs were selected for genotyping.

SNP genotyping. Genomic DNA extracted from blood or saliva samples was evaluated and quantified by Nanodrop UV-spectrometer (Thermo Fisher Scientific Inc., Wilmington, DE) and PicoGreen-based fluorometric assay (Molecular Probes, Invitrogen Inc., Carslbad, CA), and stored at -80^{o}C until analysis. To control for potential bias due to population admixture, a panel of 108 ancestry informative markers (AIMs) that have been shown to be effective in correcting this bias in case-control association studies were chosen [3]. Selected SNPs and AIMs were genotyped by Illumina GoldenGate genotyping assay (Illumina Inc., San Diego, CA) at the Genomics Facility at RPCI. Five percent duplicates and two sets of in-house trio samples were included for genotyping quality control purposes. No SNP violated Mendelian heritability. Six SNPs failed genotyping due to poor clustering or abnormal heterozygosity and were excluded. The average successful genotyping rate for each sample and each SNP was $\geqslant$99%. Three SNPs failed Hardy-Weinberg equilibrium and were excluded. As a result, a total of 145 SNPs were analyzed.

Statistical analysis. STRUCTURE program was used to estimate the proportion of European ancestry for each woman based on the genotype data of AIMs. Women with over 85% of genomic race other than the self-identified race were excluded from the analysis (n=13). Descriptive characteristics were analyzed by student t-test or chi-square test using SAS 9.2 (SAS Institute, Cary, NC). All genotype analyses were performed for AA and EA populations separately, using PLINK program if not otherwise specified. Genotypic (co-dominant) models were assumed for SNP effects. When genotype frequency of the rare homozygote was $\leqslant$5% in both populations, it was collapsed with the heterozygote (dominant model) for power considerations. In addition, recessive models were

also explored. To test if there was a linear dose-effect of the variant alleles, SNPs were coded as 0, 1 and 2 according to the copy number of the variant allele and tested using log-additive genetic models. Univariate single SNP analysis was first performed. Covariates, including age, body mass index (BMI), proportion of European ancestry, family history of breast cancer, and education, were then adjusted in multivariate logistic regression models to derive odds ratios (ORs) and 95% confidence intervals (CIs). For tagSNPs in miRNA processing genes and SNPs in miRNAs clusters, haplotype analysis was performed for those that were in LD blocks defined by the criteria by Gabriel et al [4]. Multiple comparison error was controlled by 10,000 permutations for SNP and haplotype analyses. To test whether the associations of SNPs with breast cancer differed between AA and EA women, modification effect by race was examined by including an interactive term between race and each SNP in the model based on all women and tested using likelihood ratio tests.

Results.

Differences in allele frequencies of SNPs in miRNAs and processing genes between AA and EA women. Chromosomal location and minor allele frequency (MAF) of the 145 SNPs included in the final analysis are shown in Supplementary Table S1. MAFs of 126 of the 145 SNPs in the controls were significantly different between AA and EA women ($p \leqslant 0.05$).

Associations between SNPs in miRNA genes and breast cancer risk. Top-ranked significant associations between SNPs in miRNA genes and breast cancer risk are shown in Table 2. Among AA women, rs12586258 in hsa-miR-758 was most significantly associated with risk. AA women with the A variant allele had 39% decreased risk of breast cancer (OR=0.61; 95% CI=0.43-0.88, p=0.01), compared to those without the allele. Two other SNPs, including rs2018562 in hsa-miR-513a-2 and rs5970292 in hsa-miR-105-1, were also associated with borderline significantly increased risk of breast cancer in AA women. Among EA women, we identified 7 SNPs in 7 miRNAs that were significantly associated with breast cancer risk (Table 2). However, none of the above associations remained significant after correction for multiple comparisons. Interestingly, none of the SNPs showed a significant association with breast cancer in both AA and EA populations. In fact, five SNPs showed differential associations between AA and EA populations with a p for interaction <0.05 (Table 2).

Table 2. Top ranked SNPs associated with breast cancer risk in African American and European American women

Gene/miRNA	SNP	Genotype	African American			European American			P for interaction wit race
			# Case/control	OR (95% CI)*	P	# Case/control	OR (95% CI)*	P	
hsa-miR-573	rs7696197	AA	426/362	1.00	0.45	354/364	1.00	0.03	0.01
		AG/GG	99/93	0.88 (0.64-1.22)		16/4	3.52 (1.14-10.9)		
hsa-miR-576	rs6856291	GG	281/235	1.00	0.46	278/244	1.00	0.01	0.15
		GA/AA	244/219	0.91 (0.7-1.17)		93/126	0.65 (0.47-0.9)		
hsa-miR-548a-2	rs878175	AA	139/119	1.00	0.85	274/250	1.00	0.03	0.15
		AG	385/336	0.97 (0.73-1.3)		97/119	0.7 (0.5-0.97)		
hsa-miR-608	rs4919510	CC	189/170	1.00	0.70	258/236	1.00	0.04	0.12
		CG/GG	336/285	1.05 (0.81-1.37)		113/134	0.71 (0.52-0.98)		
hsa-miR-758	rs12586258	GG	460/370	1.00	0.01	193/201	1.00	0.41	0.01
		GA/AA	64/85	0.61 (0.43-0.88)		177/169	1.13 (0.84-1.53)		
hsa-miR-544	rs10144193	AA	205/184	1.00	0.67	229/261	1.00	0.01	0.08
		AT/TT	320/270	1.06 (0.82-1.37)		141/109	1.54 (1.12-2.12)		
has-mir-487	rs1951032	GG	479/403	1.00	0.18	208/238	1.00	0.01	0.01
		GA/AA	40/47	0.73 (0.47-1.15)		120/92	1.58 (1.12-2.22)		
hsa-miR-659	rs5750504	TT	168/124	1.00	0.11	111/135	1.00	0.05	0.01
		TA/AA	356/331	0.79 (0.6-1.05)		260/235	1.37 (1.00-1.89)		
hsa-miR-513a-2	rs2018562	AA	152/161	1.00	0.05	211/209	1.00	0.84	0.11
		AG/GG	371/293	1.31 (1.00-1.72)		159/161	0.97 (0.72-1.31)		
hsa-miR-105-1	rs5970292	GG	42/55	1.00	0.04	142/137	1.00	0.59	0.04
		GA/AA	481/400	1.57 (1.02-2.41)		228/233	0.92 (0.68-1.25)		
AGO4	rs7354931	CC	476/395	1.00	0.03	368/366	1.00	0.99	0.38
		CA/AA	46/59	0.64 (0.42-0.96)		3/2	1.01 (0.15-6.89)		

AGO4	rs3820276	GG	260/258	1.00	0.03	345/340	1.00	0.53	0.19
		GC/CC	265/196	1.32 (1.03-1.71)		26/30	0.84 (0.48-1.47)		
DGCR8	rs9606241	AA/AG	486/419	1.00	1.00	340/346	1.00	0.03	0.12
		GG	39/34	1.00 (0.62-1.62)		40/24	1.80 (1.05-3.09)		
DGCR8	rs443678	GG/GA	519/446	1.00	0.48	351/332	1.00	0.02	0.64
		AA	6/8	0.68 (0.23-1.99)		20/38	0.50 (0.28-0.89)		

**Footnote:* Adjusted for age, body mass index (BMI), proportion of European ancestry, family history of breast cancer, and education.

Abbreviations: OR: odds ratio; CI: confidence interval.

In haplotype analysis, we identified two haplotypes that were significantly associated with breast cancer risk in AA women, with no associations in EA women (Table 3). The G allele of SNP rs2018562 in hsa-miR-513a-2, which is located on chromosome X in miRNA cluster (hsa-mir-513a-2/hsa-mir-506/hsa-mir-507), was associated with increased risk of breast cancer in AA women. Consistently, the G-G haplotype consisting of rs2018562 and a neighboring SNP rs5905010 in this miRNA cluster was also associated with increased risk among AA women (OR=1.54, 95% CI=1.02-2.32; p=0.03). Another haplotype consisting of two SNPs in hsa-miR-766 was also associated with decreased risk of breast cancer in AA women (Table 3).

Table 3. Haplotypes in significant association with breast cancer risk in African American women

Gene	SNPs	Haplotype	Haplotype frequency in cases	Haplotype frequency in controls	OR (95% CI)*	P
hsa-miR-766	rs5909648-rs6646439	AA	0.09	0.12	0.72 (0.54-0.97)	0.03
hsa-miR-513a-2 -hsa-miR-507	rs2018562-rs5905010	GG	0.07	0.04	1.54 (1.02-2.32)	0.03
AGO1	rs12047998-rs11263833-rs2296470-rs636832-rs595961-rs2791961-rs645383-rs595055	GCAGGCCA	0.12	0.09	1.45 (1.08-1.95)	0.02
AGO4	rs12083902-rs16822342-rs12044203-rs727005-rs636671-rs2791966-rs7354931-rs4652895-rs3820276	AAGGAAAAG	0.04	0.07	0.61 (0.40-0.90)	0.02

**Footnote:* Adjusted for age, body mass index (BMI), proportion of European ancestry, family history of breast cancer, and education.

Abbreviations: OR: odds ratio; CI: confidence interval.

Associations between SNPs in miRNA processing genes and breast cancer risk. Among AA women, two SNPs (rs7354931 and rs3820276) in AGO4 were significantly associated with breast cancer risk (Table 2). The increased risk associated with the AGO4 C allele of rs3820276 was confirmed in a long haplotype consisting of this SNP and 8 others in AGO4 (Table 3). A haplotype consisting of 8 SNPs in AGO1 was also associated with breast cancer risk in AA women. In EA women, two SNPs in DGCR8 (rs9606241 and rs44367) were significantly associated with breast cancer risk (Table 2), but there were no associations in EA women for any haplotypes.

Stratified analysis by menopausal status. After additional stratification by menopausal status, 10 SNPs that were significant in the analyses stratified only by race were significant only in either premenopausal women (rs6856291, rs12586258, rs10144193, rs1951032, rs7354931, and rs443678), or postmenopausal women (rs4919510, rs5750504, and rs9606241) (Table 4). There were also associations not previously demonstrated in the overall analyses that were significant when stratifying by menopausal stats, including 5 SNPs in 5 miRNAs in premenopausal women and 11 SNPs in 10 miRNAs and 3 SNPs in miRNA processing genes in postmenopausal women. There were also significant interactions between genotypes, risk and ancestry, with associations for 3 SNPs in premenopausal women and 7 SNPs in postmenopausal women significantly different for AA and EA women (Table 4).

Table 4. Top ranked SNPs associated with breast cancer risk stratified by menopausal status in African American and European American women

Gene/miRNA	SNP	Genotype	African American			European American			P for interaction with race
			# Case/control	OR (95% CI)*	P	# Case/control	OR (95% CI)*	P	
					Premenopausal				
hsa-miR-576	*rs6856291*	GG	165/141	1.00	0.46	169/137	1.00	0.05	0.07
		GA/AA	160/119	0.13 (0.81-1.59)		60/75	0.65 (0.42-0.99)		
hsa-miR-106b	*rs1527423*	GG	28/29	1.00	0.86	58/71	1.00	0.31	0.92
		GA/AA	130/92	1.33 (0.73-2.42)		122/92	1.69 (1.07-2.68)		
hsa-miR-548a-3	*rs11997039*	AA	293/218	1.00	0.02	227/210	1.00	0.83	0.64
		AG/GG	32/42	0.54 (0.32-0.88)		2/2	0.8 (0.10-6.25)		
hsa-miR-331	*rs11107973*	GG	51/50	1.00	0.14	72/82	1.00	0.02	0.35
		GA	157/127	1.21 (0.76-1.92)		100/99	1.18 (0.76-1.83)		
		AA	117/84	1.44 (0.88-2.36)		56/30	2.04 (1.16-3.58)		
hsa-miR-758	*rs12586258*	GG	289/211	1.00	0.01	125/110	1.00	0.87	0.04
		GA/AA	36/50	0.52 (0.33-0.84)		104/102	0.97 (0.66-1.43)		
hsa-miR-544	*rs10144193*	AA	129/101	1.00	0.86	140/154	1.00	0.01	0.04
		AT/TT	196/159	0.97 (0.69-1.36)		88/58	1.74 (1.14-2.65)		
has-miR-487	*rs1951032*	GG	300/240	1.00	0.85	127/140	1.00	0.01	0.24
		GA/AA	23/18	1.07 (0.55-2.06)		70/46	1.80 (1.13-2.85)		
has-miR-487	*rs4906032*	GG	12/17	1.00	0.19	75/91	1.00	0.05	0.90
		GA	91/72	2.00 (0.88-4.55)		123/97	1.60 (1.05-2.44)		
hsa-miR-518a	*rs4470257*	AA	169/136	1.00	0.97	180/185	1.00	0.04	0.08
		AG/GG	155/125	1.01 (0.72-1.40)		49/27	1.76 (1.04-2.99)		
hsa-miR-659	*rs5750504*	TT	102/66	1.00	0.86	74/75	1.00	0.32	0.20
		TA	138/139	0.63 (0.43-0.94)		112/103	1.13 (0.73-1.76)		
hsa-miR-105-1	*rs5970292*	GG	22/32	1.00	0.04	80/76	1.00	0.96	0.05
		GA/AA	302/229	1.88 (1.05-3.38)		148/136	0.99 (0.66-1.48)		
AGO4	*rs7354931*	CC	292/221	1.00	0.05	227/210	1.00	0.90	0.35
		CA/AA	31/39	0.6 (0.36-0.99)		2/1	0.84 (0.06-12.5)		
DGCR8	*rs443678*	GG/GA	321/255	1.00	0.45	218/182	1.00	0.003	0.43

		AA	4/5	0.60 (0.16-2.28)		11/30	0.32 (0.16-0.68)		
				Postmenopausal					
hsa-miR-578	*rs17624836*	AA	155/134	1.00	0.03	110/130	1.00	0.71	0.09
		AG/GG	44/60	0.58 (0.36-0.94)		29/27	1.12 (0.61-2.05)		
hsa-miR-598	*rs4840516*	GG	139/117	1.00	0.02	45/60	1.00	0.30	0.01
		GC/CC	60/76	0.60 (0.39-0.93)		95/97	1.30 (0.79-2.15)		
hsa-miR-598	*rs2898254*	GG	82/99	1.00	0.09	81/73	1.00	0.04	<0.01
		GA/AA	117/95	1.44 (0.95-2.18)		59/84	0.60 (0.37-0.98)		
hsa-miR-606	*rs1367290*	GG	34/17	1.00	0.01	72/87	1.00	0.41	0.01
		GA/AA	161/175	0.43 (0.22-0.82)		67/69	1.22 (0.76-1.98)		
hsa-miR-608	*rs4919510*	CC	78/76	1.00	0.90	105/102	1.00	0.03	0.13
		CG/GG	121/118	0.97 (0.64-1.48)		35/55	0.56 (0.33-0.95)		
hsa-miR-139	*rs754042*	GG	156/159	1.00	0.56	99/97	1.00	0.05	0.08
		GA/AA	32/31	1.18 (0.67-2.07)		25/42	0.55 (0.31-1.00)		
hsa-miR-624	*rs11156654*	TT	71/92	1.00	0.02	71/90	1.00	0.27	0.50
		TA/AA	128/102	1.65 (1.08-2.51)		69/67	1.31 (0.81-2.09)		
hsa-miR-196a-1	*rs718079*	AA	16/20	1.00	0.99	58/77	1.00	0.04	0.15
		AG	76/66	1.42 (0.66-3.04)		59/66	1.20 (0.72-2.01)		
		GG	107/108	1.20 (0.58-2.52)		23/14	2.46 (1.12-5.36)		
hsa-miR-659	*rs5750504*	TT	66/58	1.00	0.52	36/60	1.00	0.01	0.04
		TA	99/98	0.83 (0.52-1.33)		71/77	1.5 (0.87-2.58)		
		AA	34/38	0.85 (0.46-1.54)		33/20	2.51 (1.23-5.12)		
hsa-miR-500	*rs17174054*	AA	126/104	1.00	0.02	140/157		.	NA
		AG/GG	72/90	0.61 (0.4-0.94)		0/0	NA	NA	
hsa-miR-766	*rs5909648*	CC	142/123	1.00	0.05	121/141	1.00	0.36	0.06
		CA/AA	57/71	0.64 (0.41-0.99)		19/15	1.42 (0.67-3.00)		
XPO5	*rs11077*	CC	34/18	1.00	0.01	48/59	1.00	0.656	0.03
		CA/AA	165/176	0.43 (0.23-0.82)		92/98	1.12 (0.68-1.83)		
XPO5	*rs1106841*	CC	34/19	1.00	0.01	51/61	1.00	0.75	0.04
		CA/AA	165/174	0.44 (0.23-0.84)		89/96	1.08 (0.67-1.76)		
DGCR8	*rs9606241*	AA/AG	187/179	1.00	0.57	122/149	1.00	0.03	0.05
		GG	12/15	0.79 (0.35-1.77)		18/8	2.71 (1.11-6.62)		

**Footnote:* Adjusted for age, body mass index (BMI), proportion of European ancestry, family history of breast cancer, and education.

Abbreviations: OR: odds ratio; CI: confidence interval.

Stratified analysis by ER status. When stratifying by ER status, associations were observed that were not present in the overall analyses stratified by race only. Fourteen SNPs were associated with risk of ER-positive breast cancer in either AA or EA women, and 6 SNPs were associated with ER-negative breast cancer in either AA or EA women, all but 4 of which were not associated with breast cancer risk in overall analyses (Table 5). For 5 of these SNPs, associations were significantly different for AA and EA women (p for interaction $\leqslant$0.05). For the most significant SNP, rs107822 in hsa-miR-219, AA women carrying the G allele had almost twofold increased risk of ER-negative breast cancer compared to those carrying no G allele (OR=1.97, 95% CI=1.24-3.12), which was not observed in EA women (OR=1.54, 95% CI=0.82-2.88).

Table 5. Top ranked SNPs associated with breast cancer risk stratified by estrogen receptor status in African American and European American women

Gene/miRNA	SNP	Genotype	African American			European American			P-interaction
			# Case/control	OR (95% CI)*	P-trend	# Case/control	OR (95% CI)*	P-trend	
					ER positive				
hsa-miR-551b	*rs6771018*	AA	185/375	1.00	0.92	87/203	1.00	0.11	0.47
		AG	38/72	1.01 (0.65-1.56)		63/143	1.09 (0.73-1.63)		
		GG	3/7	0.83 (0.21-3.31)		19/23	1.99 (1.00-3.94)		
hsa-miR-573	*rs7696197*	AA	180/362	1.00	1.00	159/364	1.00	0.004	0.004
		AG/GG	47/93	1.00 (0.67-1.49)		10/4	6.14 (1.81-20.85)		
hsa-miR-576	*rs6856291*	GG	121/235	1.00	0.73	133/244	1.00	0.01	0.05
		GA/AA	106/219	0.94 (0.68-1.31)		36/126	0.56 (0.36-0.86)		
hsa-miR-218-2	*rs11134527*	GG	169/344	1.00	0.70	78/209	1.00	0.03	0.16
		GA/AA	58/110	1.08 (0.74-1.56)		91/161	1.51 (1.03-2.20)		
hsa-miR-548a-1	*rs9396886*	AA	155/312	1.00	0.98	73/193	1.00	0.02	0.09
		AC/CC	72/143	1.01 (0.71-1.42)		96/175	1.56 (1.06-2.29)		
hsa-miR-598	*rs2898254*	GG	109/226	1.00	0.60	102/189	1.00	0.03	0.12
		GA/AA	95/192	0.98 (0.69-1.37)		58/148	0.67 (0.44-0.99)		
hsa-miR-455	*rs2060133*	CC	46/73	1.00	0.14	129/247	1.00	0.03	0.59
		CG/GG	181/380	0.73 (0.48-1.11)		40/123	0.63 (0.41-0.97)		
hsa-miR-606	*rs12266981*	GG	176/381	1.00	0.03	168/369	1.00	0.99	0.97
		GA/AA	51/71	1.58 (1.05-2.38)		1/0	NA		
hsa-miR-608	*rs4919510*	CC	81/170	1.00	0.70	122/236	1.00	0.03	0.09
		CG/GG	146/285	1.07 (0.76-1.49)		47/134	0.62 (0.41-0.94)		
hsa-miR-139	*rs754042*	GG	185/376	1.00	0.82	125/223	1.00	0.02	0.14

		GA/AA	32/70	0.95 (0.60-1.50)		31/94	0.57 (0.35-0.92)		
hsa-miR-618	*rs11613504*	AA	219/445	1.00	0.30	149/301	1.00	0.05	0.07
		AG/GG	8/9	1.69 (0.63-4.50)		20/68	0.57 (0.33-0.99)		
hsa-miR-624	*rs11156654*	TT	90/221	1.00	0.17	88/209	1.00	0.14	0.08
		TA	117/187	1.56 (1.11-2.19)		66/142	1.05 (0.70-1.56)		
		AA	20/46	1.03 (0.57-1.84)		15/19	2.18 (1.04-4.58)		
hsa-miR-659	*rs5750504*	TT	72/124	1.00	0.98	50/135	1.00	0.05	0.18
		TA	100/237	0.74 (0.51-1.07)		82/180	1.28 (0.83-1.97)		
		AA	55/94	1.05 (0.67-1.64)		37/55	1.74 (1.01-3.01)		
hsa-miR-196a-1	*rs718079*	GG	108/246	1.00	0.09	26/35	1.00	0.04	0.01
		AG/AA	119/208	1.32 (0.96-1.84)		143/334	0.56 (0.32-0.97)		
				ER Negative					
hsa-miR-219	*rs107822*	GG	31/200	1.00	0.003	22/218	1.00	0.18	0.62
		GA/AA	80/252	1.97 (1.24-3.12)		23/152	1.54 (0.82-2.88)		
hsa-miR-206	*rs16882131*	GG	51/228	1.00	0.76	20/198	1.00	0.07	0.16
		GA	52/190	1.15 (0.74-1.79)		18/147	1.26 (0.64-2.48)		
		AA	8/37	0.97 (0.42-2.23)		7/23	2.91 (1.09-7.76)		
hsa-miR-608	*rs4919510*	CC	42/170	1.00	0.88	37/236	1.00	0.01	0.04
		CG/GG	69/285	0.97 (0.62-1.50)		8/134	0.36 (0.16-0.80)		
hsa-miR-758	*rs7141987*	GG	2/19	1.00	0.32	5/111	1.00	0.01	0.29
		GA/AA	108/435	2.14 (0.48-9.59)		40/252	3.83 (1.45-10.11)		
hsa-miR-494	*rs9324030*	GG	6/17	1.00	0.20	7/120	1.00	0.02	0.01
		GA/AA	102/434	0.52 (0.19-1.41)		38/247	2.82 (1.21-6.60)		
hsa-miR-495	*rs2281611*	CC	62/254	1.00	0.89	14/178	1.00	0.02	0.07
		CA/AA	49/201	1.03 (0.67-1.58)		31/192	2.21 (1.12-4.35)		

**Footnote:* Adjusted for age, body mass index (BMI), proportion of European ancestry, family history of breast cancer, and education.

Abbreviations: OR: odds ratio; CI: confidence interval; ER: estrogen receptor

KEY RESEARCH ACCOMPLISHMENTS

- Three SNPs in miRNAs and 2 in *AGO4* gene were associated with breast cancer risk in AA women.
- Seven SNPs in miRNAs and 2 in *DGCR8* gene were associated with breast cancer risk in EA women.
- Interestingly, none of the associations were found in both ancestral groups, and 5 of the associations showed significant differences by race.
- The race-specific associations were also observed after stratification by menopausal status or estrogen receptor (ER) status.
- For the most significant SNP, rs107822 in *hsa-miR-219*, AA women carrying the G allele had a twofold increased risk of ER-negative breast cancer (OR=1.97, 95% CI=1.24-3.12), with no association in EA women.

REPORTABLE OUTCOME

- **Poster presentation:** "microRNAs: Novel Breast Cancer Susceptibility Factors in Caucasian and African American Women" by Hua Zhao, DOD Era of Hope meeting, 08/2011
- **Manuscript in review:** "Genetic variants in microRNAs and breast cancer risk in African American and European American women" by Song Yao, Kelly Graham, Jie Shen, Lara E. Sucheston, Prashant Singh, Gary Zirpoli, Michelle Roberts, Gregory Ciupak, Warren Davis, Helena Hwang, Dana H. Bovbjerg, Lina Jandorf, Foluso Ademuyiwa, Karen S. Pawlish, Elisa V. Bandera, Song Liu, Christine B. Ambrosone, Hua Zhao, Carcinogenesis, 2012

CONCLUSION

To the best of our knowledge, this is the first study to investigate the role of SNPs in miRNA genes and miRNA processing genes in the etiology of breast cancer in both AA and EA women. In this study, we found prevalent differences in allele frequencies in SNPs in miRNAs, and significant associations between risk of breast cancer risk and a number of SNPs in miRNA genes, as well as in miRNA processing genes in either AA or EA women, with significant interactions for ancestry and risk for some of the associations. Further studies are needed to confirm the associations and explore the genetic basis and underlying molecular mechanisms of the associations.

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SUPPLEMENTAL

Supplementary Table S1. Chromosomal location and minor allele frequency in African American and European American women without breast cancer

Gene/miRNA	SNP	Chr.	Coordinate	Minor allele	Major allele	MAF in AA	MAF in EA	P
AGO4	rs12083902	1	36047699	A	G	0.37	0.12	<.0001
AGO4	rs16822342	1	36047775	G	A	0.24	0.05	<.0001
AGO4	rs12044203	1	36048986	A	G	0.2	0.04	<.0001
AGO4	rs727005	1	36053923	G	A	0.31	0.12	<.0001
AGO4	rs636671	1	36065797	A	G	0.34	0.18	<.0001
AGO4	rs2791967	1	36071617	G	A	0.05	0	<.0001
AGO4	rs2791966	1	36087448	A	G	0.31	0.18	<.0001
AGO4	rs7354931	1	36088518	A	C	0.05	0	<.0001
AGO4	rs4652895	1	36089158	C	A	0.25	0.18	<.0001
AGO4	rs3820276	1	36089628	C	G	0.26	0.04	<.0001
AGO1	rs12047998	1	36126956	A	G	0.25	0	<.0001
AGO1	rs11263833	1	36129199	A	C	0.11	0.02	<.0001
AGO1	rs2296470	1	36132256	G	A	0.36	0.02	<.0001
AGO1	rs636832	1	36136062	A	G	0.46	0.11	<.0001
AGO1	rs595961	1	36140367	A	G	0.23	0.18	<.0001
AGO1	rs2791961	1	36144006	G	C	0.46	0.11	<.0001
AGO1	rs645383	1	36146410	G	C	0.13	0.1	0.04
AGO1	rs595055	1	36152720	G	A	0.33	0.18	<.0001
hsa-miR-30c-1	rs16827546	1	40995476	A	G	0.17	0.04	<.0001
hsa-miR-488	rs12041859	1	175264930	G	A	0.07	0.21	<.0001
hsa-miR-488	rs10753142	1	175264991	T	A	0.26	0.45	<.0001
hsa-miR-194-1	rs3820455	1	218358110	G	A	0.07	0.06	0.74
hsa-miR-128a	rs11888095	2	136139254	A	G	0.28	0.1	<.0001

PACT	rs2059691	2	179010130	A	G	0.24	0.32	0.02
PACT	rs10930831	2	179012222	C	G	0.47	0.23	<.0001
PACT	rs2288320	2	179016748	C	A	0.18	0.19	0.78
PACT	rs3752689	2	179020859	G	A	0.48	0.23	<.0001
PACT	rs1967327	2	179022605	C	G	0.21	0.19	0.51
hsa-miR-551b	rs6771018	3	169752450	G	A	0.1	0.27	<.0001
hsa-miR-569	rs16855840	3	172307439	C	A	0.1	0	<.0001
hsa-miR-218-1	rs16869717	4	20139257	A	T	0.04	0	<.0001
hsa-miR-573	rs7696197	4	24131019	G	A	0.1	0.01	<.0001
hsa-miR-576	rs6856291	4	110629427	A	G	0.27	0.16	<.0001
hsa-miR-302d	rs13136737	4	113788584	A	C	0.14	0.48	<.0001
hsa-miR-578	rs11100610	4	166526712	A	G	0.23	0.5	<.0001
hsa-miR-578	rs17624836	4	166527025	G	A	0.14	0.12	0.12
hsa-miR-584	rs36048	5	148421906	G	C	0.35	0.21	<.0001
hsa-miR-378	rs1076063	5	149092388	A	T	0.1	0.11	0.4
hsa-miR-218-2	rs11134527	5	168127934	A	G	0.13	0.26	<.0001
hsa-miR-548a-1	rs9396886	6	18680212	C	A	0.17	0.31	<.0001
hsa-miR-219	rs107822	6	33283553	A	G	0.34	0.22	<.0001
hsa-miR-219	rs213210	6	33283802	G	A	0.09	0.07	0.53
XPO5	rs7755135	6	43598787	A	G	0.48	0.1	<.0001
XPO5	rs11077	6	43598925	A	C	0.37	0.38	<.0001
XPO5	rs7759854	6	43599419	A	G	0.12	0	<.0001
XPO5	rs2257082	6	43600556	A	G	0.17	0.29	<.0001
XPO5	rs1106841	6	43604640	A	C	0.38	0.35	<.0001
XPO5	rs7767973	6	43609811	G	A	0.49	0.07	<.0001
XPO5	rs9472070	6	43611214	A	G	0.05	0	<.0001
XPO5	rs6936089	6	43620012	G	A	0.11	0	<.0001
XPO5	rs7748977	6	43621785	G	A	0.49	0.07	<.0001
XPO5	rs34324334	6	43642996	A	G	0.01	0.06	<.0001

XPO5	rs6458342	6	43647118	A	G	0.29	0.04	<.0001
XPO5	rs699937	6	43648904	A	G	0.11	0.28	<.0001
hsa-miR-206	rs16882131	6	52116892	A	G	0.3	0.28	0.41
hsa-miR-30a	rs1358379	6	72170163	G	A	0.11	0.04	<.0001
hsa-miR-548a-2	rs878175	6	135601850	G	A	0.5	0.16	<.0001
hsa-miR-106b	rs1527423	7	99529676	A	G	0.29	0.46	<.0001
hsa-mir-595	rs12670231	7	158018099	A	G	0.41	0.46	0.003
hsa-mir-595	rs4909238	7	158018333	G	A	0.4	0.47	0.002
hsa-miR-124-1	rs531564	8	9798109	G	C	0.16	0.11	0.001
hsa-miR-598	rs4840516	8	10704745	C	G	0.19	0.38	<.0001
hsa-miR-598	rs2898254	8	10929940	A	G	0.31	0.27	0.76
hsa-miR-548a-3	rs11997039	8	105565943	G	A	0.07	0	<.0001
hsa-mir-101	rs462480	9	4840436	C	A	0.45	0.35	<.0000
hsa-miR-204	rs7861254	9	72614604	A	G	0.49	0.27	<.0001
hsa-miR-27b	rs1011784	9	96887835	G	C	0.4	0.29	0.001
hsa-miR-455	rs2060133	9	116011443	G	C	0.43	0.18	<.0001
hsa-mir-604	rs2368392	10	29874009	A	G	0.35	0.23	<.0001
hsa-miR-606	rs12266981	10	76982106	A	G	0.09	0	<.0001
hsa-miR-606	rs1367290	10	76982190	A	G	0.29	0.29	<.0001
hsa-miR-608	rs4919510	10	102724768	G	C	0.39	0.19	<.0001
hsa-miR-202	rs2185743	10	134910855	A	G	0.31	0.1	<.0001
hsa-miR-202	rs3008372	10	134910917	A	G	0.21	0.01	<.0001
hsa-miR-610	rs7944852	11	28034830	A	T	0.29	0.46	<.0001
hsa-miR-139	rs572421	11	72003858	A	G	0.26	0.26	0.53
hsa-miR-139	rs754042	11	72003923	A	G	0.08	0.15	<.0001
hsa-miR-100	rs543412	11	121528137	A	G	0.36	0.32	0.56
hsa-miR-100	rs11821130	11	121528310	G	A	0.04	0	<.0001
hsa-miR-100	rs1834306	11	121528397	G	A	0.32	0.48	<.0001
TARBP2	rs2280448	12	52183251	A	G	0.09	0.14	<.0001

TARBP2	rs7138281	12	52184341	A	G	0.03	0	<.0001
hsa-miR-196a-2	rs11614913	12	52671866	A	G	0.19	0.39	<.0001
hsa-mir-618	rs2682818	12	79853667	A	C	0.3	0.13	<.0001
hsa-mir-618	rs11613504	12	79853803	G	A	0.01	0.09	<.0001
hsa-mir-492	rs2289030	12	93752417	G	C	0.02	0.08	<.0001
hsa-miR-331	rs11107973	12	94226516	A	G	0.42	0.42	<.0001
hsa-miR-621	rs17061602	13	40282732	G	C	0.07	0.08	0.46
hsa-miR-624	rs179724	14	30553512	C	A	0.28	0.19	<.0001
hsa-miR-624	rs11156654	14	30553706	A	T	0.32	0.25	0.01
hsa-miR-337	rs1077412	14	100410387	G	A	0.04	0.25	<.0001
hsa-miR-299	rs7149786	14	100559832	A	G	0.03	0	<.0001
hsa-miR-758	rs7141987	14	100561977	A	G	0.18	0.47	<.0001
hsa-miR-758	rs12586258	14	100562301	A	G	0.08	0.28	<.0001
hsa-miR-494	rs9324030	14	100565898	A	G	0.17	0.43	<.0001
hsa-miR-495	rs2281611	14	100569702	A	C	0.26	0.31	0.08
hsa-miR-544	rs10144193	14	100584703	T	A	0.38	0.19	<.0001
has-mir-487	rs1951032	14	100588513	A	G	0.05	0.18	<.0001
has-mir-487	rs4906032	14	100588663	A	G	0.18	0.37	<.0001
hsa-miR-410	rs10144688	14	100601989	A	G	0.08	0.01	<.0001
hsa-miR-211	rs919001	15	29144430	A	G	0.47	0.43	0.25
hsa-miR-628	rs8041885	15	53452381	G	A	0.44	0.1	<.0001
hsa-miR-628	rs8041044	15	53452605	A	C	0.43	0.1	<.0001
hsa-miR-629	rs2937279	15	68158694	A	C	0.16	0	<.0001
hsa-miR-631	rs5745926	15	73433168	A	G	0.03	0	<.0001
hsa-miR-184	rs12903401	15	77289151	C	G	0.2	0.5	<.0001
hsa-miR-196a-1	rs718079	17	44064834	G	A	0.28	0.33	<.0001
hsa-miR-122a	rs17669	18	54269473	G	A	0.42	0.24	<.0001
hsa-miR-641	rs11880261	19	45480481	A	G	0.09	0.29	<.0001
hsa-miR-330	rs7252448	19	50833893	A	G	0.13	0.26	<.0001

hsa-miR-512-2	rs1968439	19	58864129	A	G	0.28	0.25	0.52
hsa-miR-515-2	rs2060230	19	58880031	G	A	0.03	0.15	<.0001
hsa-miR-526b	rs10415265	19	58889573	G	A	0.48	0.24	<.0001
hsa-miR-520d	rs2217653	19	58914976	G	A	0.07	0.24	<.0001
hsa-mir-518a	rs4470257	19	58926165	G	A	0.28	0.1	<.0001
hsa-miR-373	rs12983273	19	58983644	A	G	0.15	0.14	0.54
hsa-miR-499	rs3746444	20	33041912	G	A	0.2	0.24	0.06
hsa-miR-646	rs6027486	20	58317070	A	G	0.43	0.23	<.0001
hsa-miR-648	rs8140977	22	16843608	A	G	0.38	0.24	<.0001
hsa-miR-648	rs5992941	22	16843747	G	A	0.46	0.24	<.0001
DGCR8	rs2269724	22	18448556	A	C	0.05	0.02	0
DGCR8	rs1558495	22	18451652	C	A	0.03	0.2	<.0001
DGCR8	rs1558496	22	18451737	G	A	0.05	0.26	<.0001
DGCR8	rs35987994	22	18454006	G	A	0.01	0.06	<.0001
DGCR8	rs7291552	22	18454699	A	G	0.04	0	<.0001
DGCR8	rs9606241	22	18455858	G	A	0.28	0.29	0.74
DGCR8	rs11089328	22	18459603	G	A	0.34	0.4	0.14
DGCR8	rs1640297	22	18461852	G	A	0.39	0.46	<.0001
DGCR8	rs9606252	22	18472996	A	G	0.03	0.2	<.0001
DGCR8	rs443678	22	18473126	A	G	0.12	0.29	<.0001
DGCR8	rs1640299	22	18478359	C	A	0.37	0.47	<.0001
DGCR8	rs417309	22	18478544	A	G	0.02	0.07	<.0001
DGCR8	rs3757	22	18479331	A	G	0.14	0.25	<.0001
hsa-miR-659	rs5750504	22	36573621	A	T	0.46	0.43	0.01
hsa-let-7a-3	rs731085	22	44887429	G	C	0.24	0.3	<.0001
hsa-miR-651	rs1037521	23	8055173	A	T	0.38	0.48	<.0001
hsa-miR-500	rs17174054	23	49659688	G	A	0.25	0	<.0001
hsa-miR-374B	rs174208	23	73355366	A	C	0.13	0.08	0.005
hsa-miR-766	rs5909648	23	118664575	A	C	0.16	0.06	<.0001

hsa-miR-766	rs6646439	23	118664996	T	A	0.06	0.06	0.69
hsa-miR-424	rs757309	23	133508213	A	G	0.19	0.34	<.0001
hsa-miR-513a-1	rs7060854	23	146102550	G	A	0.36	0.02	<.0001
hsa-miR-513a-2	rs2018562	23	146114853	G	A	0.44	0.25	<.0001
hsa-miR-507	rs5905010	23	146120498	C	G	0.4	0.25	<.0001
hsa-miR-105-1	rs5970292	23	151311524	A	G	0.31	0.39	<.0001

Abbreviations: Chr., chromosome; AA: African American; EA: European American